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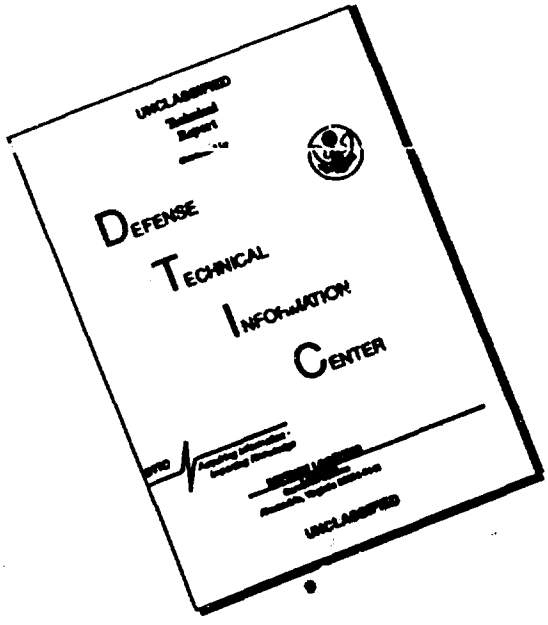

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Aerosol Inoculator for Exposure of Human Volunteers

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The performance of an aerosol inoculator for human volunteers is described in tests that used the PR8 strain of type A influenza virus and sodium fluorescein as a physical tracer. Virus recovery from the aerosols was approximately 1% and was unaffected by such variables as prolonged aerosolization, total airflow, relative humidity, or method of sampling. The recovery of sodium fluorescein from the aerosol was approximately 12% and was influenced by total airflow rates and relative humidity. With this apparatus, it should be possible to deliver reasonably predictable and measurable doses of respiratory viruses to human subjects. The design makes it possible to dismantle the inoculator into its component parts to facilitate portability.

In recent years, there has been increasing use of aerosols for the inoculation of human volunteers in the study of experimental respiratory infections. A mobile laboratory exposure unit that has been widely used in these studies was described by Griffith (1). That unit not only included the necessary aerosol equipment but also provided accessory laboratory and containment facilities for the safe handling of hazardous infectious materials. The aerosol apparatus described in this report represents an attempt to design equipment for aerosol inoculation with less hazardous infectious agents. Elimination of the accessory safety and laboratory facilities greatly reduced the cost of the equipment. To facilitate portability, the apparatus can be dismantled into three modules.

MATERIALS AND METHODS

Aerosol equipment. The aerosol equipment was designed and fabricated by the Environmental Research Corp. of St. Paul, Minn. A schematic diagram is shown in Fig. 1, and a photograph of the equipment is shown in Fig. 2. The basic aerosol tunnel is a 6-inch (15.2 cm) stainless-steel tube 60 inches in length (1.52 m). Aerosols are generated with a Collison atomizer (2) at one end of the tunnel. The human exposure ports (A and B, Fig. 1) and the sampling ports (C and D, Fig. 1) are located at the opposite end of the tunnel. The equipment consists of three modules, an air supply module, a control module, and an aerosol module (Fig. 1 and 2). The air supply module (Fig. 2, A) contains the compressors and a surge tank that supply air to the atomizer and tunnel. The control module (Fig. 2, B) contains an air dryer, humidifier, heat exchanger, and a vacuum pump. The aerosol module is made up of three units. The generation unit (Fig. 2, C) includes a Collison atomizer and an air ionizer. The second component is the aerosol tunnel unit (Fig. 2, D), and the third component, designated the exhaust unit (Fig. 2, E), contains the human exposure stations, two impinger sampling ports, a humidity sensor, constant pressure chamber, gas meters, and an exhaust blower. One impinger (C, Fig. 1) is used to sample the aerosol directly from the tunnel. The other impinger (D, Fig. 1) can be used to sample the expired air from a volunteer at station A. The expired air from each exposure station is measured through a gas meter to determine the total volume of aerosol inhaled by each volunteer. Air from all lines containing aerosol particles is filtered through absolute filters before being exhausted to the atmosphrere.

Virus. The virus used to test the performance of the aerosol equipment was the PR8 strain of type A influenza. The test pool was prepared by harvesting infected allantoic fluid of embryonated hens' eggs. The pool had a titer of $10^{7.7}$ median infectious doses (EID_{50}) per 0.1 ml for 10-day-old embryonated eggs.

Physical tracer. The physical recovery of aerosols was determined by adding sodium fluorescein to the virus suspensions used for aerosolization. In various experiments, the concentration of sodium fluorescein used in the spray suspensions ranged from 2.7 to 7.2 mg/ml. Fluorescein concentrations were measured in a Turner model 110 Fluorometer with 2A + 47B primary and 2A − 12 secondary filters.

Aerosol sampling. The aerosols were sampled with either an all-glass impinger (AGI) or a modified Shipe impinger (SI; reference 3). Each sample was 1 min in duration, which is equivalent to sampling 12.9 liters with the AGI and 10 liters with the SI. Eagle's basal medium (BME) without phenol red but containing 2% calf serum was used in the impinger

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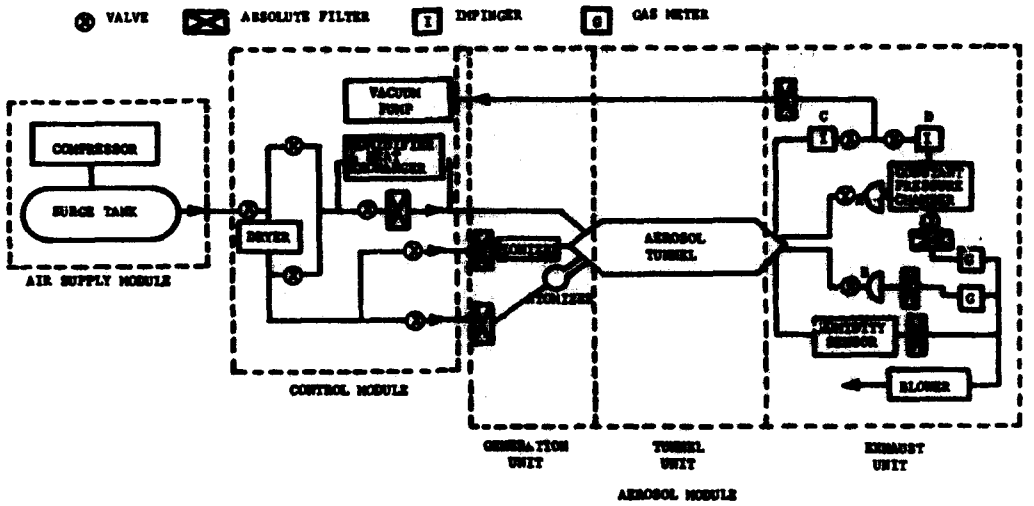

FIG. 1. *Schematic flow diagram of the aerosol inoculator.*

FIG. 2. *Photograph of the aerosol inoculator. (A) Air supply module, (B) control module, (C) aerosol generation unit, (D) aerosol tunnel, and (E) exhaust unit.*

in quantities of 20 ml for the AGI and 10 ml for the SI.

The physical and viral recoveries are expressed as percentages calculated from the experimentally determined concentration in the aerosol divided by the aerosol concentration expected based on the amount of material aerosolized.

RESULTS

Overall recovery rates. Data from several experiments involving aerosolization of many suspensions containing a variety of sodium fluorescein and influenza virus concentrations are shown in Table 1. The mean recovery of the sodium fluorescein physical tracer was approximately 12%, whereas only 1% of the virus was recovered. The disparity between the recovery rates of the physical tracer and virus is probably a reflection of the biological inactivation of the virus either during the process of aerosolization or while it is in the airborne state. From one aerosol test to another, there was considerably less variation in the physical recovery than in the virus recovery. This difference is probably attributable to the precision of the fluorescein assay, which is much greater than that of the virus assay.

Prolonged aerosolization. Under certain experimental conditions, it is necessary to generate aerosols over a relatively long period of time. It was of interest to determine whether prolonged aerosolization would alter the recovery of physical particles or their biological content. Tests were performed by initiating the aerosolization process and continuing to generate an aerosol over a 31-min period. At various intervals, impinger samples were taken for a 1-min period and assayed for both sodium fluorescein and virus content. The results of several tests are shown in Table 2. There appears to be no significant change in the mean physical or virus recovery rates with time. When the values from the first

TABLE 1. *Overall aerosol recovery rates*

Expt no.	Aerosol test	Per cent recovery	
		Physical	Virus
I	1	12.3	
	2	11.3	
	3	14.9	
	4	16.9	0.29
	5	12.8	0.27
II	1	12.4	0.57
III	1	12.0	
	2	11.9	
	3	12.8	
	4	14.0	0.57
	5	12.8	2.66
IV	1	11.7	1.36
VI	1	10.4	0.61
VII	1	10.0	0.51
	2	10.6	1.23
	3	17.2	1.16
VIII	1	16.0	1.39
IX	1	9.4	1.01
	2	9.6	2.32
Mean		12.13	0.95

four sampling periods were compared with those obtained from the last four, there were no statistically significant differences.

In each aerosol test, samples of the spray suspension in the atomizer were taken immediately before and after aerosolization. These samples were assayed for both sodium fluorescein and virus content. The intervals of aerosolization ranged from 6 to 51 min. The results (Table 3) showed no significant increase in virus concentration over the time periods tested. There was, however, an increase in fluorescein concentration as a result of prolonged atomization. The increased concentration of fluorescein with atomization for 16 min or longer was significantly higher than that seen with less than 8 min.

Total airflow. The airflow through the aerosol inoculator can be varied by regulating the flow of secondary air that is used to mix with the aerosol. Aerosol recovery determinations were made by using 200 and 100 liters per min secondary airflows, giving total airflow rates of 208 and 108 liters per min, respectively. The mean per cent recovery values are given in Table 4. Physical recovery at the 108 liters per min airflow was significantly higher than at 208 liters per min. There was no statistical difference between the two airflow rates in terms of virus recovery.

Relative humidity. The moisture content of the aerosol can be controlled by directing various proportions of the secondary air through a dryer or humidifier to obtain a desired level of humidity. In two separate experiments testing four aerosols

TABLE 2. *Per cent physical and viral recovery from aerosols during prolonged aerosolization*

Time[a] of aerosolization (min)	Mean per cent recovery	
	Physical[b]	Virus[c]
1–2	11.15	0.36
4–5	11.14	0.77
7–8	12.06	0.42
10–11	11.87	0.59
15–16	10.05	1.45
20–21	14.88	0.69
25–26	16.06	0.91
30–31	15.56	1.95

[a] Shape impinger samples were taken for 1 min during the indicated interval after aerosolization began.
[b] Mean of four tests.
[c] Mean of two tests.

TABLE 3. *Concentration of virus and sodium fluorescein in spray suspensions before and after aerosolization*

Aerosol test	Spray interval (min)	Difference in	
		Log virus titer[a]	Sodium fluorescein conc[b]
1	6	0.4	0
2	6	0	0.7
3	6	0	0.5
4	7	−0.2	0.2
5	7	0.2	0.6
6	8	0.4	0.2
7	16	0.1	1.3
8	20	0	1.8
9	27	0	0.9
10	31	0.8	1.2
11	31	0.2	0.7
12	32	0.2	2.3
13	32	0.1	1.8
14	48	−0.2	2.7
15	51	0.8	1.5

[a] EID_{50} per 0.1 ml (concentration before minus concentration after). Values obtained from 8 min or less were not significantly (5% level) different from those from 16 min or greater.
[b] Milligrams per milliliter (concentration before minus concentration after). Values obtained from 8 min or less were significantly (1% level) lower than those obtained from 16 min or greater.

TABLE 4. *Effect of total airflow on physical and viral recovery percentages*

Airflow (liters/min)	Mean per cent recovery	
	Physical	Virus
208	11.98[a]	3.62[b]
108	14.74[a]	1.00[b]

[a] Statistically different at the 5% level by F-test analysis.
[b] Not statistically different at the 5% level.

TABLE 5. *Effect of relative humidity (RH) on physical and viral recovery percentages*

RH (%)	Mean per cent recovery	
	Physical	Virus
20–21	9.84[a]	3.01[c]
50	12.40[b]	1.71[c]
77–98	9.12[a]	1.69[c]

[a] Not significantly different at the 5% level.
[b] Significantly different at the 1% level.
[c] Not significantly different at the 5% level.

at each relative humidity (RH), physical tracer and viral recovery values were determined for 20, 50, and 70% RH. The results in Table 5 showed no effect of humidity on virus recovery, but significantly more fluorescein dye was recovered at 50% RH than at either extreme.

Comparison of samplers. In two experiments involving four aerosols each, the sampling capability of an SI was compared with that of Porton-type AGI. The results given in Table 6 showed no significant differences in the physical tracer or viral recovery of aerosols by the two impingers.

Other variables. Other factors that were considered in these experiments included the effect of the ionizer and the volume of fluid used in the impinger. The ionizer was included in the design to help counteract aggregation or precipitation by static electrical forces. Operation of the apparatus with the ionizer on or off did not influence aerosol recovery.

The sampling efficiency of the SI was tested with 10 or 5 ml of impinger fluid. The volume of the fluid in the impinger had no influence on the recovery of either fluorescein or virus.

All of the above tests were done with a Collison atomizer that had an internal baffle cylinder in place. In an attempt to increase aerosol recovery, a second series of tests was conducted with the baffle cylinder removed. In these tests, the average recovery of fluorescein was 16.33% (Table 7). This recovery was significantly higher than the 12.13% (Table 1) obtained with the baffle in place.

TABLE 6. *Comparison of Shipe and all-glass impingers as aerosol samplers*

Impinger	Mean per cent recovery	
	Physical	Virus
Shipe.....	11.37[a]	0.52[b]
All-glass.....	10.53[a]	0.50[b]

[a] Not significantly different at the 5% level.
[b] Not significantly different at the 5% level.

TABLE 7. *Physical recovery of aerosols after removal of the baffle cylinder from the Collison atomizer*

Aerosol test	Mean per cent recovery
1	19.2
2	19.1
3	12.8
4	15.0
5	14.4
6	15.5
Overall mean	16.33

DISCUSSION

The aerosol equipment described in this study meets the basic requirements for use as an aerosol inoculator for the study of human respiratory disease. The average recovery from aerosols of the physical tracer, sodium fluorescein, was 12%. The average recovery of virus was only 1%, suggesting biological inactivation of the virus in the aerosols. Because the virus assay was less precise than the assay of fluorescein, there was a much higher variance in the virus recovery percentages than in the physical recovery percentages.

Within the limits of the tests included in this study, virus recovery was unaffected by such variables as: (i) prolongation of aerosolization, (ii) total airflow, (iii) RH, or (iv) type of impinger used. Total airflow rate and RH did influence physical recovery percentages.

Aerosolization for periods of 16 min or longer from the same spray suspension resulted in an increased concentration of fluorescein in the suspension. This might be expected because of diluent evaporation that takes place in the Collison, a reflux-type atomizer. Although a similar trend in concentration was seen with the virus, a statistically significant difference was not noted, probably because of the high variance in the observations. Removal of the baffle cylinder from the atomizer increased the physical recovery

from the aerosol. Virus recovery was not tested in these experiments, but it can be expected that similar increases in virus recovery would be found.

Based on the overall virus recovery values given in Table 1, it was possible to calculate human doses that would have been presented under similar conditions of exposure. These calculations showed that, if an experimenter attempted to deliver a dose of 100 ID_{50}, the actual values would vary from 12 to 277 (75% tolerance limits and 0.95 confidence). The mean human dose would fall between 62.5 and 149 (0.95 confidence). The doses were found to be normally distributed in the square root transform, and the above statistics were calculated from the transformed values and decoded to the original scale.

With this aerosol equipment, it should be possible to deliver reasonably predictable doses of viruses to human subjects. The apparatus can be conveniently moved from one location to another. Variables such as total airflow and humidity can be readily controlled and measured. The apparatus is equipped to meter the volume of air expelled by the volunteer during exposure, and it is even possible to sample the expired air to determine quantity of inoculum that is exhaled and, therefore, estimate the retained dose.

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LITERATURE CITED

1. Griffith, W. R. 1964. A mobile laboratory unit for exposure of animals and human volunteers to bacterial and viral aerosols. Amer. Rev. Resp. Dis. 89:240–249.
2. Henderson, D. W. 1952. An apparatus for the study of air bourne infection. J. Hyg. 50:53–68.
3. Public Health Monograph No. 60. 1969. Sampling microbiological aerosols. U.S. Public Health Service, no. 686.